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DEVELOPMENT OF SALINITY-TOLERANT WHEAT RECOMBINANT LINES FROM A WHEAT DISOMIC ADDITION LINE CARRYING A *THINOPYRUM JUNCEUM* CHROMOSOME

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Three *Triticum aestivum* L. × *Thinopyrum junceum* (L.) A. Löve partial amphidiploids (2n = 8x = 56; 21″ **ABD** + 7″ $\mathbf{E^b/E^e}$) and 11 derived disomic addition lines (2n = 44) were screened for salt tolerance in hydroponic solutions. One addition line (AJDAj5, 21″ **ABD** + 1″ $\mathbf{E^b}$) had salt tolerance comparable to that in partial amphidiploids. It was crossed to a wheat line having the Ph^I allele from *Aegilops speltoides* Tausch to induce homoeologous pairing. F_2 plants were subjected to salt screening and advanced to 30 F_3 families, which were screened again. Four F_3 lines were more tolerant than AJDAj5 when screened in a final electrical conductivity of 42 dS/m. Because one of the four lines was sterile, only three lines were further verified for their salinity tolerance and were cytologically and molecularly analyzed. These lines were translocation lines with 42 chromosomes having tiny fluorescent hybridization signals detected at interstitial positions of less condensed chromosomes using the genomic *in situ* hybridization technique. Amplified fragment length polymorphism analyses revealed the presence of very few (ca. 4%) putative markers specific to the $\mathbf{E^b}$-chromosome addition line. These lines also had from 2% to 14% of markers specific to the *Ph* inhibitor line and a few new AFLP markers that were not found in the two parental lines and the common wheat background, cv. Chinese Spring. Two recombinant lines were more salt tolerant than either parent, while the third one was as tolerant as either parent, which was more tolerant than Chinese Spring. The former two lines are valuable germplasm for breeding salt-tolerant wheat cultivars.

Keywords: AFLP, FISH, GISH, Ph^I, salt tolerance, amphiploid, addition line, translocation line, *Triticum aestivum*, wheat, *Thinopyrum junceum*, *Aegilops speltoides*.

Introduction

Although variation in salt tolerance was observed among barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L. and *Triticum turgidum* L.) varieties, none of these cultivated crops was as tolerant as some wild annual and perennial Triticeae species (Dewey 1960; McGuire and Dvořák 1981). The most salt tolerant are species belonging to the genus *Thinopyrum,* composed of the diploid *Thinopyrum bessarabicum* (Savul. and Rayss) A. Löve and *Thinopyrum elongatum* (Host) D. Dewey; tetraploid *Thinopyrum junceiforme* (A. Löve and D. Löve) A. Löve; hexaploid *Thinopyrum junceum* (L.) A. Löve and *Thinopyrum intermedium* (Host) Barkworth and D. Dewey; octoploid *Thinopyrum runemarkii* A. Löve; and decaploid *Thinopyrum ponticum* (Podpera) Liu and Wang (Dewey 1960; McGuire and Dvořák 1981; Forster et al. 1988). Some of these species have been crossed to wheat, and studies on derived addition or substitution lines showed that salt tolerance in these perennial grasses is controlled by multiple genes on several chromosomes (Dvořák et al. 1988; Forster et al. 1988; Dubcovsky et al. 1994; Zhong and Dvořák 1995). Therefore, transfer of salt tolerance by introducing alien genes into wheat is more complicated than transfer of pest resistance that is usually controlled by a single gene.

To transfer a single gene from an alien chromosome into wheat, wheat scientists often rely on methods that induce recombinations through suppression or removal of activity of the *Ph1* gene located on the 5**B**L of wheat. The production of a *Ph* inhibitor line (Chen et al. 1994) and the *ph1b* mutant (Sears 1977) was done to increase homoeologous pairing and to promote chromosomal recombinations. The *ph1b* mutant, in which the *Ph* gene was deleted by x-ray irradiation, has been successfully utilized to effect translocation of genes for pest resistance (Liang et al. 1979; Kibirige-Sebunya and Knott 1983; Koebner and Shepherd 1985; Islam and Shepherd 1992). In spite of its potential usefulness, the *Ph* inhibitor line (Ph^I) has not been successfully used to produce any translocation line (B. S. Gill, personal communication).

Partial amphidiploids and disomic addition lines have been synthesized from the cross *T. aestivum* × *T. junceum* (Charpentier 1992). Although *T. junceum* has the genome formula $\mathbf{E^bE^bE^bE^bE^eE^e}$ (Liu and Wang 1993; Wang et al. 1995) and is known to be salt tolerant, these lines have not been screened

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for salt tolerance. In this article, we report the identification of one disomic addition line that has usable salt tolerance. This article also reports the first successful gene transfer using *Ph*^{I} to induce genetic recombinations. However, this is only the first in a series of papers to provide molecular and cytogenetic evidence for the genetic introgression involving the disomic addition line AJDAj5 and the Ph^{I} line in the three identified wheat translocation lines, two of which are more salt tolerant than either parent. Subsequent papers will report physiological characterization of the salinity tolerance in these three lines and molecular mapping of the recombinant chromosome or chromosomes using restricted fragment length polymorphism (RFLP), simple sequence repeat (SSR), sequence tagged site (STS), and amplified fragment length polymorphism (AFLP) markers.

Material and Methods

A greenhouse screening for salt tolerance was conducted in 1995 at the USDA-ARS Forage and Range Research Laboratory in Logan, Utah. Three partial amphidiploids and 11 disomic addition lines obtained from A. Charpentier (Institut National de la Recherche Agronomique, France) were grown in pure sand with one plant per cone-tainer. The Ray Leach "Cone-tainer"[5] (Stuewe and Sons, Corvallis, Oreg.) has a diameter of 3.8 cm and a depth of 21 cm. Chinese Spring wheat, the recurrent parent of these backcross derivatives, was used as the control. Starting at the four-leaf stage, 18 plants per line were subjected to salinity stress through serial soakings of a rack of 84 cone-tainers, each containing one plant, in salt solutions to saturate the sand. The electrical conductivity (EC) of the salt solution was measured with a conductivity meter and was increased weekly. The initial EC was 6 dS/m and was increased by an increment of six per week for six or seven times until the final EC reached 42 or 48 dS/m. The NaCl and $CaCl_2 \cdot H_2O$ concentrations were 0.92 and 2.25 g/L, respectively, for an EC of 6 dS/m. The final concentrations were 2.83 and 19.78 g/L of NaCl and $CaCl_2 \cdot H_2O$, respectively, for an EC of 42 dS/m. The salts were mixed in a complete nutrient solution with both macro- and micro-nutrients. The diluted nutrient solution without the sodium and calcium salts had an EC of 2 dS/m. The cone-tainers were immersed in the salt solution for 10 min each, three times a week. Between treatments, plants were only sprinkled with water to keep them from wilting. When the leaves of the Chinese Spring had turned completely yellow, the surviving plants with green leaves in the more salt-tolerant lines were counted and transplanted into 6-in pots containing a normal potting medium.

To determine whether the salt-tolerant addition line AJDAj5 is also carrying the 5**E**^{b} (=5**J**) chromosome of *Thinopyrum bessarabicum* as the salt-tolerant disomic addition line developed by Forster et al. (1988), these two lines were analyzed along with Chinese Spring, *T. bessarabicum*, amphidiploid of Chinese Spring × *T. bessarabicum*, and disomic addition lines having 1**E**^{b}, 2**E**^{b}, 4**E**^{b}, 5**E**^{b}, and 7**E**^{b} (Zhang et al. 2002), using 565 decamers from Operon Technologies (Alameda, Calif.) as single primers for random amplified polymorphic DNA (RAPD) analyses (Wei and Wang 1995). The three translocation lines were also included in these RAPD analyses.

The salt-tolerant disomic addition line AJDAj5 was crossed with the Ph^{I} wheat line provided by B. S. Gill of Kansas State University. F_2 and F_3 derivatives of the cross were screened for salt tolerance as described above. The F_5 selfed progenies (lines 4909, 4910, and 4911) of three identified translocation lines (lines 2407, 2457, and 2461) were tested in 2000 for salt tolerance along with Chinese Spring, Ph^{I}, AJDAj5, and Yecora Rojo (the salt-tolerant standard cultivar for California) at the USDA-ARS Salinity Laboratory in Riverside, California. The detailed analyses of salt tolerance in these three translocation lines will be reported separately (C. M. Grieve, M. C. Shannon, and R. R.-C. Wang, unpublished data). In addition, these three lines were also observed for salt tolerance under the field conditions in La Paz, Baja California Sur, Mexico, in collaboration with A. Mujeeb-Kazi of the International Maize and Wheat Improvement Center (CIMMYT).

Chromosome numbers in salt-tolerant lines or individuals were determined from root-tip chromosome counts using the procedures of Mujeeb-Kazi and Miranda (1985). Presence of chromosomes or chromosome segments of *Thinopyrum junceum* in common wheat was detected by genomic *in situ* hybridization (GISH) using the DNA of *Pseudoroegneria stipifolia* (Czern. ex Nevski) Á. Löve (2n=14, **St** genome) as a probe and that of Chinese Spring as a block. The GISH technique followed the procedures of Wang and Zhang (1996) except the probe : block ratio was increased to 1 : 120–150 to eliminate cross hybridization on all wheat chromosomes. Genomic DNA from *P. stipifolia* was labeled with biotin-16-dUTP using the BioNick Labeling System (Life Technologies, Rockville, Md.). Ninety nanograms of labeled DNA were added as the probe in the hybridization solution with 120–150 times the amount of autoclaved genomic DNA of wheat (*Triticum aestivum* cv. Chinese Spring, **ABD** genome), and 50 times the amount of autoclaved salmon sperm DNA were added as the block. Hybridization signals were amplified and detected using fluorescein isothiocyanate-avidin D and biotinylated goat anti-avidin D (A2001 and BA-0300, respectively, Vector Laboratories, Burlingame, Calif.). The chromosomes were counterstained by 0.02%–0.05% propidium iodide (PI) in vectorshield mounting medium (Vector Laboratories).

To further confirm the genetic introgression, AFLP was used to identify molecular markers incorporated from the two parental lines, Ph^{I} and AJDAj5. DNA was extracted from young leaves of Chinese Spring, Ph^{I}, AJDAj5, and the three translocation lines using the Dneasy Plant Maxi Kit (Qiagen, Valencia, Calif.). The AFLP procedures were those of Vos et al. (1995). Selective amplification primers were 48 combinations of six *Eco*RI (with ACA, ACC, ACG, ACT, AGC, or AGG at their 3′ end) and eight *Mse*I (with CAA, CAC, CAG, CAT, CTA, CTC, CTG, or CTT at their 3′ end) primers (GIBCO BRL AFLP Pre-amp Primer Mix I kit, Life Technologies). The polymerase chain reaction (PCR) was performed using a GeneAmp9700 (PE Applied Biosystem, Foster City, Calif.) and *Taq* DNA polymerase (Promega, Madison, Wis.). The *Eco*RI selective amplification primers were 5′ labeled with 6-carboxyfluorescein (6-FAM). The AFLP amplification products and GS400-ROX internal lane size standards (PE Applied Bio-

[5] Trade names are included for the benefit of the reader and imply no endorsement or preferential treatment of the listed products by the USDA.

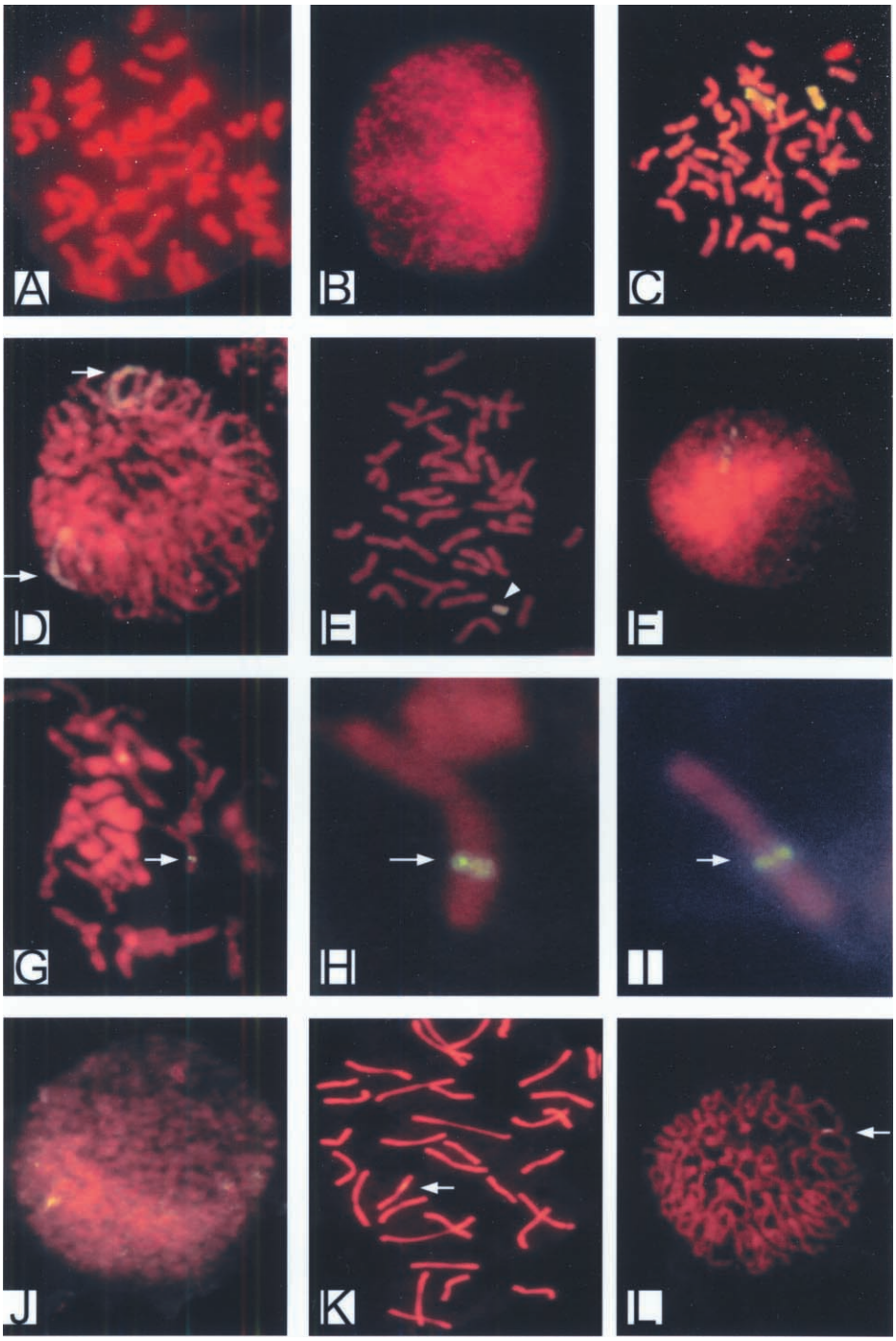

Fig. 1 Genomic *in situ* hybridization photomicrographs showing (*A*) a metaphase cell of Chinese Spring having all chromosomes uniformly stained in red by propidium iodide (PI) without yellow fluorescent signal, (*B*) an interphase mitotic cell of Chinese Spring, (*C*) a metaphase mitotic cell of the salt-tolerant *Triticum aestivum* × *Thinopyrum junceum* disomic addition line AJDAj5 with a pair of yellow fluorescent *Thinopyrum* chromosomes, (*D*) an early prophase cell of AJDAj5 with two wishbone-shaped alien chromosomes (arrow), (*E*) a metaphase cell of a monotelosomic (arrowhead) addition line derived from AJDAj5, (*F*) the interphase cell of the same plant as in 1*E*, (*G*) a metaphase cell of the translocation line 2407 having interstitial yellow fluorescent hybridization signals (arrow), (*H*) a close-up of the metaphase chromosome in *G* having interstitial yellow fluorescent hybridization signals (arrow), (*I*) another metaphase chromosome in line 2407 having interstitial yellow fluorescent hybridization signals (arrow), (*J*) an interphase cell of line 2407 with tiny hybridization signals on relaxed chromatin, (*K*) part of a metaphase cell in the translocation line 2457 with one chromosome displaying an interstitial hybridization site (arrow), and (*L*) an early prophase cell of the line 2457 with a tiny fluorescent hybridization signal (arrow). Yellow fluorescent fluorescein isothiocyanate signals are indicative of hybridization sites between the E-genome chromatin and the probing biotin-labeled genomic DNA of *Pseudoroegneria stipifolia* (**St**-genome).

systems) were fractionated and analyzed by GeneScan 3.1 on an ABI3100 DNA sequencer (PE Applied Biosystems). GeneScan files were subsequently aligned and scored for the presence and absence of bands in 1-bp bins using Genographer (Benham et al. 1999). The AFLP markers were classified based on qualitative and quantitative differences of bands in the two parental lines and their common background, Chinese Spring (table 1).

Results

Salt-Tolerant French Introductions

Three partial amphidiploids among the 14 French lines had varying degrees of salt tolerance, while only one (AJDAj5) of the 11 addition lines had a comparable tolerance to the tolerant partial amphidiploid (table 2). This disomic addition line has a pair of chromosomes (fig. 1*C*) from *Thinopyrum junceum* (2n = 42, haplome $\mathbf{E^bE^bE^e}$). The alien chromosome has an easily visible small satellite on the short arm (data not shown) and produces the $\mathbf{E^b}$ specific random amplified polymorphic DNA (RAPD) marker $OPF03_{1296}$ (table 3), which is absent in the $\mathbf{E^e}$ genome. However, it differed from the 5$\mathbf{E^b}$ addition line by having RAPD fragments specific to other $\mathbf{E^b}$ chromosomes (table 3). Additionally, the 5$\mathbf{E^b}$ addition line has compacted spikelets at the top portion of spikes, whereas AJDAj5 has spikes with lax spikelets along the whole spike.

Salt-Tolerant Derivatives of AJDAj5 × Ph^I

The salt-tolerant AJDAj5 was crossed with the wheat carrying the Ph^I gene. The hybrid (2n = 43) was allowed to self, and 195 F_2 plants were screened for salt tolerance. Thirty F_3 families from 103 tolerant F_2 plants were randomly selected and screened again. Four families showed a higher survival rate than 75%, suggesting that the salt tolerance genes were not on the alien chromosome in a monosomic state. Three (lines 2407, 2457, and 2461) of these four lines produced seeds, and their selfed progenies were euploids with 42 chromosomes. Since all plants in line 2606 were sterile, its chromosome number could not be determined.

Table 1

Classification of AFLP Markers Based on the Phenotype in Chinese Spring (CS), Ph Inhibitor Line (Ph^I), and the *Thinopyrum junceum* Disomic Addition Line AJDAj5

Specificity	Allele type	Variation	Phenotype		
			CS	Ph^I	AJDAj5
CS specific	Dominant	Qualitative	+	−	−
		Quantitative	++	+	+
CS specific	Null	Qualitative	−	+	+
		Quantitative	+	++	++
Ph^I specific	Dominant	Qualitative	−	+	−
		Quantitative	+	++	+
Ph^I specific	Null	Qualitative	+	−	+
		Quantitative	++	+	++
AJDAj5 specific	Dominant	Qualitative	−	−	+
		Quantitative	+	+	++
AJDAj5 specific	Null	Qualitative	+	+	−
		Quantitative	++	++	+

Note. Phenotype is expressed as presence or absence of band or by difference in band intensity: − = absence of band; + = presence of band; ++ = presence of more intense band.

Table 2

Salt Tolerance (Mean of 18 Replications, with Standard Deviations Given in Parentheses) in *Triticum aestivum* × *Thinopyrum junceum* Derivatives Introduced from France

Line	2n	Rating on May 2 with EC = 42 dS/m	Rating on May 11 with EC = 48 dS/m
AJDAj1	44	0.83 (0.79)	0 (0)
AJDAj2	44	0.06 (0.24)	0 (0)
AJDAj3	44	0.28 (0.57)	0 (0)
AJDAj4	44	0.28 (0.46)	0.06 (0.24)
AJDAj5	44	1.94 (0.24)	1.00 (0.77)
AJDAj6	44	0.11 (0.32)	0 (0)
AJDAj8	44	0.72 (0.75)	0.06 (0.24)
AJDAj11	44	0.44 (0.51)	0 (0)
HD3505	44	1.28 (0.67)	0.11 (0.32)
HD3508	44	1.00 (0.69)	0 (0)
HD3515	44	0.56 (0.78)	0 (0)
AJAP3	56	1.11 (0.32)	0.67 (0.59)
AJAP4	56	1.56 (0.51)	1.22 (0.65)
AJAP8	58	1.78 (0.55)	1.56 (0.70)

Note. Rating: 0 = dead plant; 1 = plant weakened but still alive; 2 = green and healthy plant. This greenhouse trial was conducted at the USDA-ARS Forage and Range Research Laboratory in Logan, Utah, in 1995.

Salt tolerance of the F_5 families 4909, 4910, and 4911 (derived from 2407, 2457, and 2461, respectively) was tested at the USDA-ARS Salinity Laboratory (table 4). Both parental lines, AJDAj5 and Ph^I, had a higher salt tolerance than Chinese Spring, a moderately salt-tolerant line itself. Two of the three putative translocation lines, lines 4909 and 4910, were more salt tolerant than the parental lines. However, differences between lines 4909 and 4910 were detectable in the four criteria of salt tolerance (table 4). Under the field conditions in La Paz, the salt tolerance in lines 4909 and 4910 was close to that in the cultivar Kharchia 65 (from India), the most salt-tolerant wheat in the world (A. Mujeeb-Kazi, personal communication).

GISH Study

There was no fluorescent signal in mitotic cells of Chinese Spring under the 1 : 120–150 probe : block ratio used in this study (fig. 1*A*, 1*B*). The *Thinopyrum* chromosomes in AJDAj5 were easily detectable by GISH (fig. 1*C*, 1*D*). Although detection of *Thinopyrum* chromatin in the three salt-tolerant recombinant lines by GISH using total genomic DNA of *Pseudoroegneria stipifolia* as a probe was difficult, especially with condensed metaphase chromosomes, observations of tiny hybridization signals on chromatin (or less condensed chromosomes) in either interphase or metaphase nuclei of mitotic cells (fig. 1*G*–1*L*) were possible. GISH results indicated that lines 2407 and 2457 contain a short *Thinopyrum* chromatin in a wheat chromosome (cf. fig. 1*J* and 1*L* to 1*F*), making them interstitial recombinant lines.

Table 3

RAPD Analyses of *Thinopyrum bessarabicum* Specific Markers in AJDAj5, Chinese Spring × *T. bessarabicum* Amphidiploid, and Five Disomic Addition Lines Derived from the Amphidiploid Having Different E^b Chromosomes

RAPD markers	AJDAj5	Amphidiploid	$1E^b$	$2E^b$	$4E^b$	$5E^b$	$7E^b$
$OPB03_{410}$	+	+	+	−	−	−	−
$OPB18_{750}$	+	+	+	+	+	+	+
$OPF03_{1296}$	+	+	+	+	+	+	+
$OPO17_{600}$	+	+	+	+	+	+	+
$OPC07_{290}$	+	+	+	+	+	+	+
$OPF07_{550}$	+	+	−	−	+	−	−
$OPJ01_{500}$	+	+	+	−	−	−	−
$OPB09_{680}$	+	+	−	−	+	−	−
$OPP04_{350}$	−	+	−	+	−	−	−
$OPD12_{500}$	−	+	−	+	−	−	−
$OPD12_{900}$	+	+	+	+	−	+	−
$OPH11_{500}$	−	+	−	−	−	+	−
$OPF12_{290}$	+	+	−	−	−	−	−
$OPC03_{330}$	−	+	−	−	−	−	−

Note. AJDAj5 and the $5E^b$ addition line differ by six RAPD markers (in boldface).

Molecular Confirmation of Introgression in Recombinant Lines

Results of AFLP analyses are summarized in table 5. Of the 48 primer combinations used, 43 produced a total of 3146 bands from Chinese Spring, Ph^I, AJDAj5, and the three translocation lines. There were six dominant- and 12 null-allele AFLP markers specific to Chinese Spring that were not found in Ph^I and AJDAj5. The Ph^I had 118 dominant- and 127 null-allele markers that were absent in both Chinese Spring and AJDAj5. AJDAj5 had 146 dominant- and 14 null-allele markers. In addition, the three translocation lines had a total of 16 dominant- and 12 null-allele new (*de novo*) markers that were not present in Chinese Spring, Ph^I, and AJDAj5. The three translocation lines differed from each other by having a combination of AFLP markers from Ph^I and AJDAj5 as well as *de novo* ones, some unique and some shared. Some examples of AFLP markers are presented in figure 2. Lines 4909, 4910, and 4911 had 19, 26, and 4, respectively, qualitatively unique AFLP markers coming from the Ph^I parent (table 6). However, they had 9, 7, and 9, respectively, qualitatively unique markers of AJDAj5 origin.

Discussion

AJDAj5 has a pair of chromosomes from *Thinopyrum junceum* with easily visible satellites, similar to those of 6**B** of wheat, on the short arm (data not shown). *Thinopyrum junceum* has the genome formula $E^bE^bE^bE^eE^e$ (Liu and Wang 1993; Wang et al. 1995). Because the addition line produced the E^b specific RAPD marker $OPF03_{1296}$ that is absent in the E^e genome, the alien chromosome must be an E^b-genome chromosome. The chromosome in the E^e genome that has a small but visible satellite belongs to homoeologous group 6, whereas those that have a large satellite belong to group 5, according to Dvořák et al. (1984). Based on differences in spike morphology described earlier and RAPD markers (table 3), AJDAj5 is certainly different from the disomic $5E^b$ addition line of Forster et al. (1988), which was derived from hybrids of wheat and the diploid *Thinopyrum bessarabicum* (2n = 14, E^b). The $5H^{ch}$ disomic addition line that contains the group 5 chromosome from *Hordeum chilense* and the $5E^b$ disomic addition line have been widely used as a source of salt tolerance genes in other germplasm enhancement programs (Jones and Gorham 1986; Forster et al. 1990). AJDAj5 has RAPD markers specific to $1E^b$, $4E^e$, and $3E^b$ or $6E^b$ chromosomes instead of those specific to $5E^b$ (table 3). Because *T. junceum* is a hexaploid species, it is not unreasonable to assume that its chromosomes had undergone structural changes so that they are different from those in the diploid *T. bessarabicum*. The

Table 4

Percent Reductions for Different Traits in Wheat Varieties and Germplasm Lines Grown at Four Different Concentrations of Salt Solution, Compared with the Respective Control

Wheat lines and EC (dS/m)	Plant height	No. heads	Straw per plant	Grain per plant
Yecora Rojo:				
8	12	29	32	35
12	20	44	46	48
18	35	59	67	80
22	40	74	86	83
Chinese Spring:				
8	9	34	41	14
12	15	15	44	10
18	24	62	84	70
22	34	70	90	73
Ph^I:				
8	8	6*	27*	0*
12	12	19	42	0*
18	26	43*	60*	11*
22	30	62	86	50*
AJDAj5:				
8	6	44	48	16
12	18	46	54	50
18	24	50*	76	13*
22	30	68	88	55*
4909:				
8	13	24	36	0*
12	12	33	48	0*
18	16	12*	67	0*
22	28	44*	84	0*
4910:				
8	7	0*	17*	0*
12	10	15	41	0*
18	20	0*	61*	0*
22	20*	0*	78*	0*
4911:				
8	6	20*	29*	4*
12	10	25	42	18
18	25	45*	73	32*
22	32	61	85	58*

Note. Control: EC = 2.7 dS/m. This three-replication trial was conducted at the U.S. Salinity Laboratory, Riverside, California, in 2000. Straw per plant is vegetative tissue per plant; grain per plant is seed weight produced per plant.

* Significantly better (at 0.05 level) than Chinese Spring.

Table 5

Number and Percent of Both Qualitative and Quantitative AFLP Markers of Ph^I, AJDAj5, and *de novo* Origin in Translocation Lines 4909, 4910, and 4911 Using 43 Primer Sets That Amplified 3146 Bands

Marker source and allele type[a]	Number of marker	Number (%) of AFLP marker[b] present in the line		
		4909	4910	4911
Ph^I:				
Dominant	118	13 (11.02)	16 (13.56)	3 (2.54)
Null	127	12 (9.45)	14 (11.02)	2 (1.57)
AJDAj5:				
Dominant	146	6 (4.11)	5 (3.42)	7 (4.79)
Null	14	9 (64.29)	8 (57.14)	10 (71.43)
New:				
Dominant	16	1 (6.25)	10 (62.50)	11 (68.75)
Null	12	1 (8.33)	6 (50.00)	5 (41.67)

[a] Presence of an AFLP marker band is regarded as the dominant allele, whereas the absence of the marker band represents the null allele.

[b] These markers include both qualitative and quantitative markers. The former differs by presence and absence; the latter differs by band intensity.

alien chromosome in AJDAj5 could have segments from the 1E^b and 4E^b chromosomes in addition to the satellite of 6E^b. Being different from the 5E^b disomic addition line, AJDAj5 would represent an additional useful germplasm for transferring salt tolerance into wheat.

In efforts to transfer useful genes from a disomic addition line or a disomic substitution line, either ph1b mutant or null-5**B** lines of Chinese Spring can be used to remove the effect of the *Ph* gene on homoeologous pairing. However, using these stocks requires tedious cytogenetic analyses of hybrid progenies in the germplasm enhancement scheme (Wang et al. 1977; Liang et al. 1979). Availability of the Ph^I line reduces the complexity of the procedures by immediately suppressing the *Ph* allele in the F_1 hybrids of an addition line and the Ph^I line, allowing homoeologous pairing to occur in the F_1 hybrids. Therefore, we chose this method to promote genetic introgression between the *Thinopyrum* chromosome in AJDAj5 and its homoeologous wheat chromosomes.

Among 30 F_3 families derived from salt-tolerant F_2 individuals, four families appeared to be probable translocation lines with better salt tolerance than Chinese Spring. One line was sterile, possibly because of a duplication-deficient translocation resulting from interchanges between nonhomoeologous chromosomes. The other three families had reasonably high fertility and the euploid chromosome constitution (2n = 42).

The F_5 progenies of the three families were more salt tolerant than Chinese Spring, and two parental lines were also more tolerant than Chinese Spring. Only two (4909 and 4910) of the three translocation lines were more tolerant than either parent (table 4), suggesting that lines 4909 and 4910 might have acquired different salt tolerance genes from both parents, while line 4911 acquired salt tolerance from one parent only.

Ph^I was derived from BC_2F_3 from a cross between Chinese Spring and *Aegilops speltoides* accession TA 1786, which has the *Ph^I* allele that is epistatic to the *Ph* allele of wheat (Chen et al. 1994). Certain *A. speltoides* accessions must have better salt tolerance than common wheat. In this study, we found the Ph^I line to be as salt tolerant as AJDAj5. Because *A. speltoides* is the most closely related species to the donor of the **B** genome of durum and bread wheats, the Ph^I line is considered to have the same **ABD** genome constitution as bread wheat. GISH analysis would not be able to detect chromosomal interchanges between chromosomes of *A. speltoides* and common wheat. However, our AFLP data (table 5) indicate an 8.4%

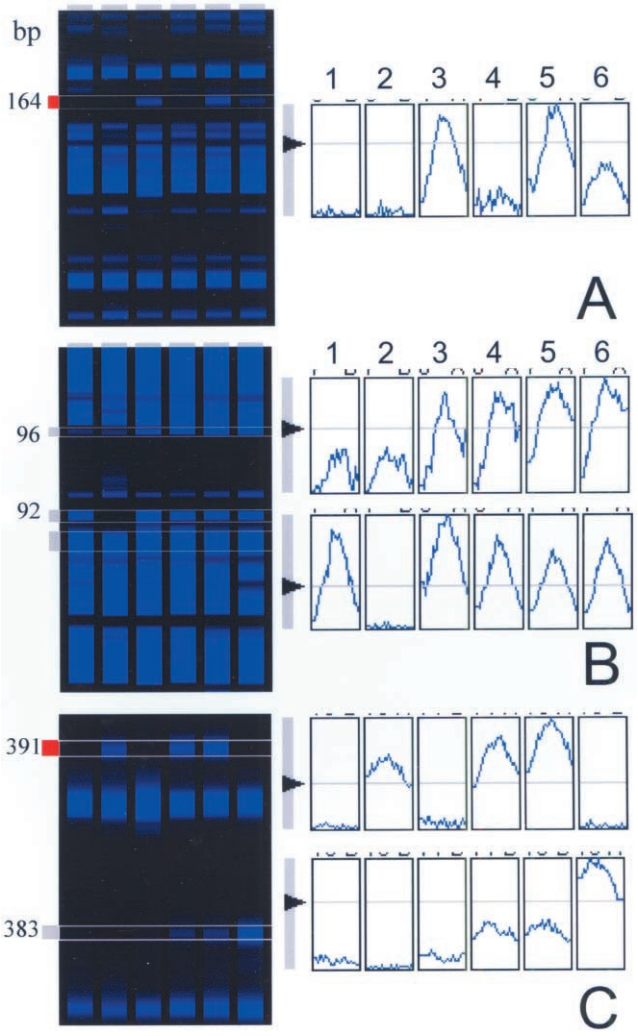

Fig. 2 Genographer-generated gel images (left) and thumbnail graphs (right) of amplified fragment length polymorphism markers: (*A*) E35M50.164, (*B*) E35M62.96 (top) and E35M62.92 (bottom), and (*C*) E41M59.391 (top) and E41M59.383 (bottom). E35M50.164 is absent in Chinese Spring (lane 1) and Ph^I (lane 2) and present at the same intensity in AJDAj5 (lane 3) and translocation line 4910 (lane 5) but less intensely in lines 4909 (lane 4) and 4911 (lane 6). E35M62.96 is present at two different levels in Chinese Spring/Ph^I (lanes 1, 2) and AJDAj5/translocation lines (lanes 3–6). E35M62.92 is a null-allele marker specific to Ph^I (lane 2). E41M59.391 is a Ph^I specific marker present in lines 4909 and 4910. E41M59.383 is a *de novo* AFLP marker absent in Chinese Spring, Ph^I, and AJDAj5 but present in three translocation lines at two different intensities.

Table 6

Qualitative AFLP Markers in Three Salt-Tolerant Wheat Translocation Lines Derived from Hybrids between the Ph^{I} Line Containing Chromatin from *Aegilops speltoides* and the AJDAj5, a Disomic Addition Line Carrying a *Thinopyrum junceum* Chromosome

AFLP marker	Chinese Spring	Ph^{I}	AJDAj5	W4909	W4910	W4911
E36M47.075	−	+	−	−	+	−
E36M49.253				+	+	−
E36M59.336				−	+	−
E36M61.158				−	+	−
E36M61.255				−	+	−
E37M47.137				−	+	+
E37M47.152				−	+	+
E37M48.092				+	−	−
E37M49.216				+	−	−
E37M59.228				−	+	−
E37M61.166				+	+	−
E37M62.210				−	+	−
E38M47.090				−	+	−
E38M60.285				+	+	−
E38M61.298				−	+	−
E40M49.066				−	−	+
E41M47.316				+	+	−
E41M49.326				+	−	−
E41M59.391				+	+	−
E41M60.381				+	+	−
E41M61.076				+	−	−
Total markers				10	16	3
E35M62.226	+	−	+	+	−	+
E36M59.311				−	−	+
E36M61.115				−	−	+
E36M62.168				−	+	+
E37M50.220				+	−	+
E38M47.189				−	−	+
E38M47.218				−	−	+
E38M60.170				−	−	+
E41M49.129				−	−	+
E41M59.197				−	−	+
E41M62.359				−	−	−
Total markers				9	10	1
E35M59.115	+	+	−	−	+	−
E35M62.212				−	−	+
E35M62.223				−	−	−
E36M61.152				−	−	−
E36M61.326				−	+	−
E37M59.082				−	−	−
E38M61.296				−	+	−
Total markers				7	4	6
E36M50.164	−	−	++	−	++	+
E36M47.202	−	+	++	++	++	++
E36M47.292	−	+	++	++	++	++
E36M49.075	+	++	−	+	+	−
Total markers				2	3	3

$([6 + 12 + 118 + 127] \div 3146)$ genetic difference between Ph^{I} and Chinese Spring, whereas AJDAj5 differs from Chinese Spring by 5.7% $([6 + 12 + 146 + 14] \div 3146)$. With 3146 AFLP marker bands amplified from 21 wheat chromosomes and one *Thinopyrum* chromosome, there would be an average of 143 markers per chromosome. Since AJDAj5 is unlikely to contain other *Thinopyrum* chromatin than the single pair of **E^{b}** chromosomes, most, if not all, of the 146 dominant-allele markers specific to AJDAj5 could be attributed to this alien chromosome, and the 14 null-allele markers might be attributed to losses of priming sites on some Chinese Spring chromosomes during the development of AJDAj5. The unusually high number of null-allele markers specific to the Ph^{I} line suggests the presence of two **S**-genome chromosomes, or their equivalents, from *A. speltoides*. These *A. speltoides* chromosomes or chromosome segments carried the null-alleles of those corresponding Chinese Spring alleles. Because of free recombination between chromosomes of the **S** genome and the **B** genome, it

would be hard to rapidly eliminate these null-alleles through backcrossing.

The introgression of a very small segment of the $\mathbf{E}^{b}$ chromosome from AJDAj5 into wheat chromosomes in translocation lines 4909, 4910, and 4911 was indicated by results from both GISH and AFLP analyses (figs. 1, 2; tables 5, 6). The presence of $\mathbf{E}^{b}$ genome-specific RAPD marker $OPF03_{1296}$ in AJDAj5 and its absence in the three translocation lines (data not shown) also corroborate the shortness of the transferred alien chromatin. This RAPD marker is dispersed along all seven chromosomes of the $\mathbf{E}^{b}$ genome (Zhang et al. 1998). The absence of this sequence in all three translocation lines indicates that only the chromosome segment between two $OPF03_{1296}$ sites from the $\mathbf{E}^{b}$ chromosome was transferred into one, but possibly different, recipient wheat chromosome in the three translocation lines. If the 146 dominant-allele AFLP markers specific to AJDAj5 are evenly distributed over the entire length of the $\mathbf{E}^{b}$ chromosome, possibly no more than 5% of the genetic material of the alien chromosome was introgressed into wheat chromosomes in translocation lines 4909, 4910, and 4911 (table 5). This could account for the difficulty in detecting the alien transfer in translocation lines 4909, 4910, and 4911 by the use of total genomic DNA as a probe in GISH analyses. Using highly specific marker sequences for the transferred chromosomal segment as probes in FISH analyses may improve the hybridization and detection efficacy.

Although we identified the presence of AJDAj5 and Ph^{I} specific AFLP markers in translocation lines 4909, 4910, and 4911, we do not know which of these markers are potentially useful in marker-assisted selection for salt tolerance. Mapping of known RFLP and SSR markers of wheat along with those AFLP markers associated with salt tolerance in translocation lines 4909, 4910, and 4911 will be carried out later using the doubled haploid mapping populations being developed, through pollination with maize pollen, by A. Mujeeb-Kazi at CIMMYT. That would provide a more detailed characterization of recombinant chromosomes and mapped positions of quantitative trait loci controlling salinity tolerance in these lines.

This is the first report providing both cytological and molecular evidence for the successful genetic introgression of alien chromatin into wheat chromosomes using the Ph^{I} allele. This is also the first report on the salt tolerance of the Ph^{I} wheat line. Our data also suggest that salinity tolerance in Chinese Spring, Ph^{I}, and AJDAj5 is controlled by different genes and that translocation lines 4909 and 4910 have probably accumulated genes from both parents, resulting in greater salt tolerance than that present in either parent. Because Kharchia 65 is not likely to have salt tolerance genes coming from the genus *Thinopyrum*, the recombinant lines 4909 and 4910 will be useful germplasm in wheat breeding programs by providing additional genes for pyramiding salinity tolerance genes.

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